

AN EXPERIMENTAL ANALYSIS OF THE DISCONTINUOUS RESPIRATION OF THE CECROPIA SILKWORM¹

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The breathing of insects has been an object of recurrent interest since the seventeenth century when Malpighi (1669) published his celebrated description of the tracheal system of *Bombyx mori* and correctly ascribed to it the function of respiration. Though other early investigators, including Lyonet, Réaumur, Bonnet, and Spallanzani, recognized that the spiracles were the sites where air made entrance into the tracheal tubes, they believed that expiration occurred elsewhere, through the mouth, or anus, or invisible pores in the skin (*cf.* Wigglesworth, 1931). It was Treviranus (1816–20) who first suggested that the spiracles provide the pathway for both inspiration and expiration—a view later amplified by the British physician, George Newport (1836). Newport was especially concerned with the respiration of lepidopterous pupae. Though apparently aware that breathing motions are undetectable in such pupae, he nevertheless supposed that the ventilation of the tracheae was due to active respiratory movements in which “nearly all the muscles and nerves of each segment are brought into consentaneous action as the muscles of the chest and ribs in vertebrated animals” (page 546).

Thomas Graham in 1833, having just discovered his law of gaseous diffusion, expressed a view contrary to that of Newport and suggested that simple physical diffusion of gases through the tracheal system might suffice for the respiration of most insects. Nearly one hundred years elapsed before experimental proof of this hypothesis was forthcoming. Then in a series of ingenious experiments of a type for which he was justly famous, Krogh (1920) showed that simple diffusion of oxygen and carbon dioxide through the spiracles and tracheae could more than suffice for the respiration of insects lacking respiratory movements. The essential elements of Krogh's theory have stood the test of time and have been most recently examined in detail by Thorpe and Crisp (1947).

On the basis of this long evolution of present thought concerning the mechanism of insect respiration, there was no theoretical basis for anticipating a series of puzzling observations which we first encountered five years ago in measurements of the respiratory quotients of diapausing pupae of the Cecropia silkworm. The R.Q. in these studies was found to undergo large and recurrent fluctuations as a function of time, in that prolonged periods of very low quotients were punctuated by more transient periods of very high quotients. It soon became evident that the source of the variability was a discontinuous release of carbon dioxide from the insects' tracheal system—a phenomenon which, we subsequently learned, had

¹ This study was aided by a grant to C. M. Williams from the U. S. Public Health Service, by an Institutional grant to Harvard University from the American Cancer Society, and by the Sage and Sackett Funds of Cornell University.

already been described by Punt in 1944 and attributed to a corresponding rhythm in the opening of the spiracular valves.

In the absence of measurements of oxygen uptake, Punt had apparently presumed that the uptake of oxygen, like the output of carbon dioxide, was discontinuous and synchronized with the opening of the spiracular valves. Consequently, Punt's work failed to reveal the true proportions of the respiratory paradox which became apparent only when the intake of oxygen was found to be *continuous* (Schneiderman, 1953; Schneiderman and Williams, 1953a, 1953b). This implies that during the period which separates the carbon dioxide "bursts," the spiracles permit the steady entry of oxygen while opposing the steady exit of carbon dioxide—a finding which appeared to be inconsistent with any simple theory of the diffusional transport of respiratory gases *via* the spiracle. Consequently, the discontinuous release of carbon dioxide has been studied in detail during the past five years. The present report considers a series of experiments which clarify the nature of the discontinuous respiration and provide a factual basis for theoretical interpretation.

MATERIALS AND METHODS

1. *Experimental animals*

The present investigation is based on approximately 1000 respiratory measurements performed on a total of 300 larvae, pupae, and developing adults of the giant silkworm, *Platysamia cecropia*. In occasional experiments pupae of other closely related saturniids were employed; namely, *Samia walkeri*, *Antheraea mylitta*, *Tecla polyphemous*, and *Actias luna*.

2. *Manometric methods*

The respiratory exchange in most experiments was determined manometrically according to techniques previously described (Schneiderman and Williams, 1953a). Measurements were performed by the "direct manometric method" (Umbreit *et al.*, 1949) in 45-cc. cylindrical vessels equipped with venting plugs and adaptors for use with standard Warburg manometers. In studies of oxygen uptake the carbon dioxide output was absorbed on two corrugated strips of filter paper, moistened with a total of 0.3 to 1 cc. of 1 N NaOH or KOH and placed above and below the animal. Determinations were performed over periods that averaged about 8 hours, the excursion of the manometer being recorded at intervals of from 5 minutes to several hours as dictated by the rate of oxygen uptake. For the purpose of measuring carbon dioxide output, the filter paper was moistened with 0.3 to 1 cc. of 0.01 N HCl; manometer readings were then recorded over periods that averaged about 24 hours at intervals of from 2 to 30 minutes depending on the rate of carbon dioxide output. In experiments of long duration, the vessels were periodically removed from the bath, opened, and equilibrated with air for a few moments, to insure a constant gaseous environment. Although the metabolic rate of individual diapausing pupae is substantially constant over periods of at least a week, measurements of oxygen uptake and carbon dioxide output were ordinarily made within 12 hours of one another.

In one series of experiments, the gas exchange was measured volumetrically

in a capillary respirometer. This and other special methods will be discussed in the appropriate sections. Each type of experiment, unless otherwise noted, was performed on a minimum of five animals.

THE CYCLE OF CARBON DIOXIDE RELEASE

Figure 1 illustrates a typical experiment descriptive of the cyclic release of carbon dioxide. Here the respiratory exchange of a *Cecropia* pupa was measured over two successive 18-hour intervals. During the first period of 18 hours, sodium

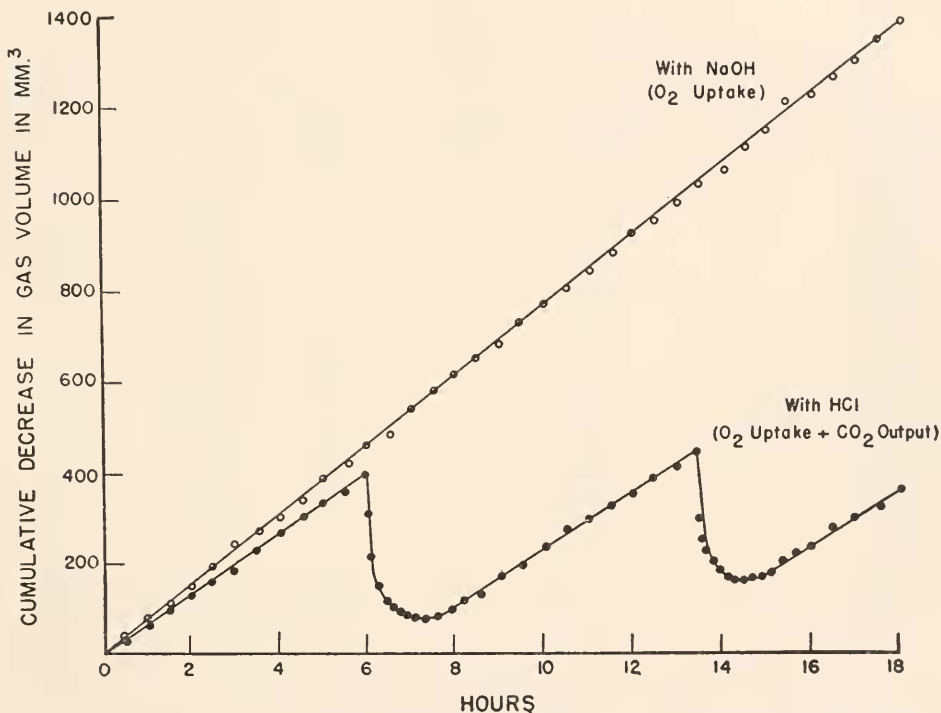


FIGURE 1. The gas exchange of a *Cecropia* pupa over two successive 18-hour intervals. The upper curve (open circles) records measurements performed in the presence of alkali; for the lower curve (solid circles) the alkali was replaced by acid. An apparent decrease in the rate of oxygen uptake followed by a slight increase was usually recorded at intervals which corresponded with the time of the carbon dioxide bursts. This result is an artifact due to inability of the alkali to absorb the large burst of CO_2 as rapidly as it evolved. By employing a larger filter paper surface for CO_2 absorption, this artifact could be almost completely eliminated.

hydroxide was placed in the vessel to absorb the carbon dioxide and thereby permit measurements of oxygen consumption; the alkali was then replaced by acid and the measurements repeated. As recorded in Figure 1, in the presence of alkali the gas volume of the respirometer decreased steadily during the entire 18-hour period, save for occasional extremely minor variations. But when acid was present to prevent the absorption of carbon dioxide, the gas volume exhibited

tremendous discontinuities. In the experiment plotted, a steady decrease in volume is evident until the sixth hour. There then occurs an abrupt increase in volume which evidently signals a rapid evolution of gas from the insect.

Results of this type permit one to construct an over-all picture of the kinetics of oxygen uptake and carbon dioxide output, as shown in Figure 2. Here we see that the rate of carbon dioxide output over the 18-hour period was discontinuous, while the rate of oxygen uptake was apparently continuous. At approximately 6-hour intervals the animal gave off a burst of carbon dioxide; meanwhile, between

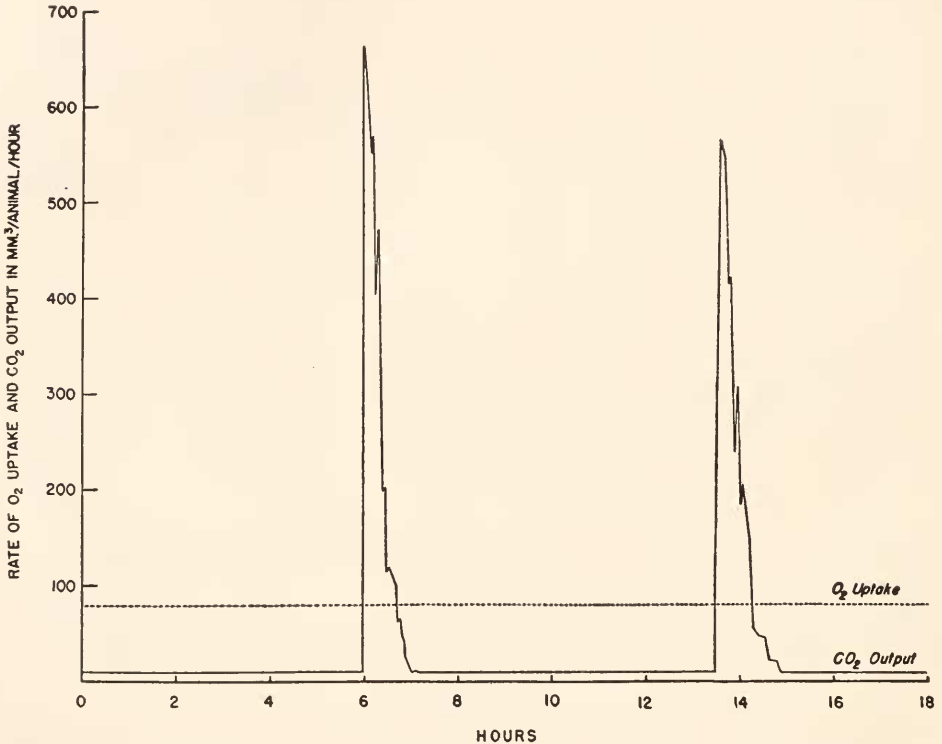


FIGURE 2. Rates of oxygen consumption and carbon dioxide output of a diapausing pupa over an 18-hour interval. The curve describing the carbon dioxide output was calculated from the data presented in Figure 1 by the "direct method" of Warburg.

successive bursts only 5 per cent of the total carbon dioxide was released. The carbon dioxide output during the first 12 minutes of the burst was 280 times larger than during a corresponding period between bursts. In this particular pupa, which weighed 5 grams and displaced about 5 cc., the average burst volume was 500 mm³; *i.e.*, a volume of carbon dioxide far exceeding the total volume of the insect's tracheal system (see Discussion).

Under constant experimental conditions, each individual repeats the recurrent cycle of carbon dioxide release day after day with only minor variations. But, among different individuals, considerable variations are encountered in the mag-

nitude and timing of the cycles. For any particular animal the quantitative aspects of the cycle can be resolved into four parameters: (1) the average rate of oxygen consumption or carbon dioxide output over the whole cycle (*i.e.*, the metabolic rate); (2) the rate of continuous carbon dioxide release between bursts; (3) the duration of the cycle as measured from the end of one burst to the end of the succeeding burst (or its reciprocal, the frequency of bursts); and (4) the burst volume.

Table I records all of these parameters for a representative sample of the several hundred pupae on which respiratory measurements were performed. The

TABLE I

The cycle of carbon dioxide release of 14 normal diapausing pupae at 25° C.

Animal no.	Weight (grams)	Over-all rate of O ₂ consumption (mm ³ /gm. live wt./hr.)	Over-all rate of CO ₂ output (mm ³ /gm. live wt./hr.)	Interburst rate of CO ₂ output (mm ³ /gm. live wt./hr.)	Average cycle length (hours)	Average burst volume/animal (mm ³)
578	4.24	7.8	5.8	0.03	9.7	242
675	6.62	8.9	7.6	0	6.5	309
558(1)	4.55	9.2	8.3	2.4	14.3	386
558(2)	4.74	9.3	7.8	0.74	9.4	314
		9.5	7.8	0.42	9.3	325
557	4.86	10.1	9.1	0.29	11.9	505
		10.4	9.3	0.16	10.8	406
559(1)	4.52	10.9	7.5	0	3.8	129
581	4.22	11.6	8.5	0.57	6.7	226
559(2)	4.99	13.0	12.8	3.7	7.8	314
		13.8	12.9	4.4	7.4	300
562	5.15	14.4	10.9	5.2	13.8	384
555	5.11	15.4	13.0	1.6	6.8	379
		15.4	12.5	2.8	7.3	332
576	5.19	15.6	11.5	2.1	2.0	94
672	3.80	23.4	21.8	5.8	2.6	156
673	3.79	25.0	19.3	6.3	3.7	165
674	4.30	32.5	30.5	7.8	3.5	267
Average*	4.72±.71	14.8±7.22	12.5±6.86	2.6±2.69	7.4±4.01	271±107.9

* In the case of the four individuals for which duplicate determinations are recorded, the average of the pairs of determinations was used in computing the over-all averages.

values for each individual represent the average of at least two cycles. In the case of four individuals, duplicate determinations obtained after intervals of about a week have been included to illustrate the regularity and persistence of the cycles.

THE EFFECTS OF PUPAL MASS AND METABOLIC RATE ON THE CYCLE OF CARBON DIOXIDE RELEASE

1. *Effects of pupal mass*

Among the various factors which were studied in relation to the cyclic release of carbon dioxide, the first to be examined in detail was the insect's size or mass. As illustrated in the typical results recorded in Table II, it was noted as a general

principle that large pupae give off larger and less frequent bursts than do smaller pupae of similar metabolic rate.

2. *Effects of the absolute level of metabolism*

Figure 3 describes the relation between metabolic rate and the four parameters of the cycle of carbon dioxide release in a homogeneous series of pupae of approximately the same mass (4 to 6 grams each). To obtain animals with very high metabolic rates, pupae with integumentary injuries were employed (see section 4 below). Each value is an average obtained over at least two cycles.

TABLE II
The cycle of carbon dioxide release of five paired groups of normal diapausing pupae
(The individuals in each pair have similar rates of oxygen consumption but different weights)

Animal no.	Weight (grams)	Rate of O ₂ consumption (mm ³ /gm. live wt./hr.)	Interburst rate of CO ₂ output (mm ³ /gm. live wt./hr.)	Average cycle length (hours)	Average burst volume (mm ³)	Average burst volume/gm. (mm ³)
562	4.81	8.7	0.46	4.4	179	37
675	6.62	8.9	0	6.5	309	47
718(9)	3.35	12.8	7.5	6.5	52	16
559	4.99	13.0	4.4	7.4	300	60
593	3.47	15.3	5.1	1.7	58	17
555	5.11	15.4	1.6	6.8	379	74
594	2.70	23.4	6.6	1.5	68	25
672	3.80	23.4	5.8	2.6	156	41
591	2.70	28.5	12.6	0.60	23	9
674	4.30	32.5	7.8	3.5	267	63

As Figures 3A and B reveal, cycle length and burst volume vary inversely with metabolic rate; *i.e.*, pupae with high metabolic rates are characterized by frequent small bursts. Indeed, it will be noted that when metabolic rate exceeded about 60 mm³/gm. live wt./hr., many animals gave off their carbon dioxide continuously; however, a few pupae with rates of oxygen uptake as high as 200 mm³/gm. live wt./hr. showed occasional irregular bursts.

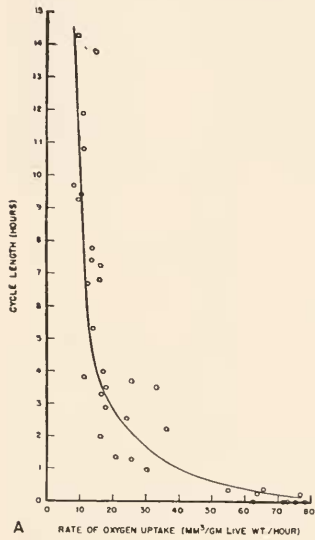
Figure 3C demonstrates the direct proportionality between cycle length and burst volume. This correlation was also seen within the cycles of individual pupae. Figure 3D shows that as the metabolic rate increases, the interburst rate of carbon dioxide output increases.

3. *Effects of temperature-induced changes in metabolism*

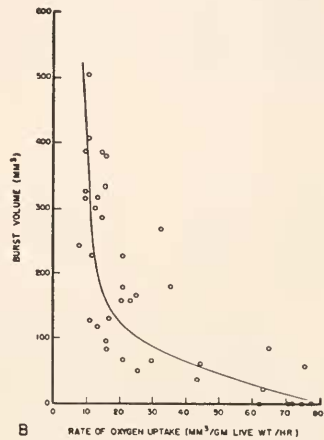
Results obtained on two groups of diapausing pupae are illustrated in the data summarized in Table III. In five individuals the rates of oxygen consumption

and carbon dioxide release were first measured at 25° C.; the insects were then equilibrated for one day at 10° C. and the respiratory measurements repeated at the low temperature. In seven other individuals the measurements were performed only at 10° C. after equilibration at this temperature for two days.

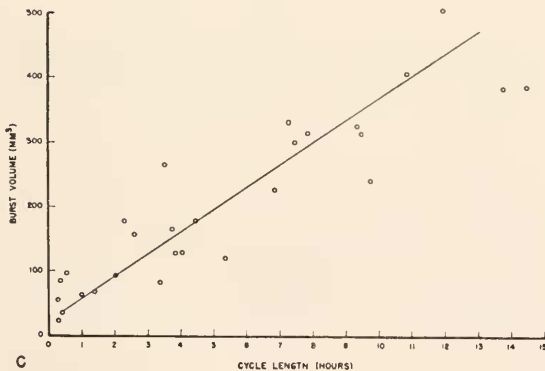
It will be observed that low temperature greatly amplifies the cyclic character of carbon dioxide release. Thus, the individual with the lowest metabolism among those in Table III (No. 572) showed, at 10° C., a calculated burst frequency of



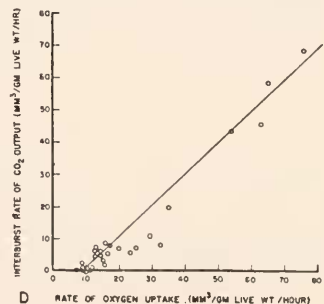
A



B



C



D

FIGURE 3A. The relation between cycle length and metabolic rate for a series of *Cecropia* pupae weighing between 4 and 6 grams.

FIGURE 3B. The relation between burst volume and metabolic rate for the same pupae as in 3A.

FIGURE 3C. The relation between burst volume and cycle length for the same pupae as in 3A.

FIGURE 3D. The relation between the interburst rate of carbon dioxide output and metabolic rate for the same pupae as in 3A.

TABLE III

The effect of temperature on the cycle of carbon dioxide release of diapausing pupae

Animal no.	Weight (grams)	Rate of O ₂ consumption (mm ³ /gm. live wt./hr.)		Interburst rate of CO ₂ output (mm ³ /gm. live wt./hr.)		Interburst ratio*		Average cycle length (hours)		Average burst volume (mm ³)	
		25°	10°	25°	10°	25°	10°	25°	10°	25°	10°
718(3)	3.87	13.2	2.69	5.7	0.98	50	41	5.3	75** (>58.5)	116	373
718(1)	4.90	20.0	3.42	6.7	0.55	43	23	1.35	25	67	242
718(2)	4.04	15.8	3.62	8.2	1.51	57	42	3.3	20	82	166
718(5)	4.42	16.5	3.62	5.3	0.68	41	21	4.0	23	130	292
718(7)	3.85	16.8	5.30	9.4	1.61	100	40	0	5	0	72
572	4.36	—	1.41	—	0.014	—	1.3	—	160** (>27)	—	764
683	5.25	—	1.57	—	0.061	—	5.0	—	50	—	—
690	5.17	—	1.68	—	0.056	—	4.3	—	65** (>28.5)	—	420
695	5.21	—	1.86	—	0.13	—	8.8	—	50	—	—
706	6.51	—	1.93	—	0.39	—	26.0	—	50	—	—
580	4.80	—	2.01	—	0.58	—	28.0	—	26	—	178
566	5.46	—	3.0	—	0.73	—	27.0	—	17	—	158

* Interburst ratio = $\frac{\text{Interburst rate of CO}_2 \text{ output}}{\text{Average overall rate of CO}_2 \text{ output}} \times 100$. This ratio indicates approximately the percentage of carbon dioxide given off continuously.

** In these experiments only one burst was observed and the cycle length was estimated by assuming an over-all R.Q. of 0.78 and a constant interburst rate of CO₂ output. The actual observed minimum values for the cycle length are recorded in parentheses.

one per 160 hours; *i.e.*, about one burst per week. At still lower temperatures to which pupae are normally exposed during winter, one may predict even less frequent release of carbon dioxide—perhaps once in several weeks.

In addition to decreasing the burst frequency, low temperature greatly de-

TABLE IV

The effect of injury on the respiration of six diapausing pupae (Measurements performed at 10° C.)

Animal no.	Weight (grams)	Rate of O ₂ consumption (mm ³ /gm. live wt./hr.)		Interburst rate of CO ₂ output (mm ³ /gm. live wt./hr.)		Average cycle length (hours)		Average burst volume (mm ³)	
		Pre-injury	Post-injury	Pre-injury	Post-injury	Pre-injury	Post-injury	Pre-injury	Post-injury
718(3)	3.87	2.7	6.8	0.98	0.49	75	6.7	373	84
718(11)	2.80	3.0	5.7	0.61	0.72	33	7.0	160	95
718(1)	4.90	3.4	8.4	0.55	2.1	25	6.7	242	121
718(5)	4.42	3.6	8.7	0.68	0.79	23	5.8	367	115
718(2)	4.04	3.6	8.6	1.51	3.2	20	2.0	166	28
718(7)	3.85	5.3	11.2	1.61	2.7	5	2.0	89	59

pressed the volume and rate of release of carbon dioxide between bursts; simultaneously the burst volume increased two- to three-fold. One individual (No. 718 (7)) showed continuous carbon dioxide release at 25° C. and cyclic release at 10° C.

4. *Effects of injury-induced increase in metabolism*

Diapausing pupae respond to integumentary injury by a prompt and spectacular increase in oxygen uptake of as much as 8-fold, which then persists for one to several weeks (Schneiderman and Williams, 1953a). By exploiting the injury effect, it was possible to induce major variations in the metabolism of individual animals and to judge the influence on cyclic carbon dioxide release.

Table IV records results obtained in this manner on a series of six animals. The gas exchange was first measured at 10° C. Each pupa was then injured by excising the tip of its abdomen, the resulting defect being sealed with melted

TABLE V

The cycle of carbon dioxide release of an animal during the larval-pupal transformation
(An. No. 595; weight = 2.97 gm.)

Days after pupation	-2	1	2	4	6	10	18	34
Rate of O ₂ consumption (mm ³ /gm. live wt./hr.)	200	131	92	62	47	48	30	15
Interburst rate of CO ₂ output (mm ³ /gm. live wt./hr.)	158	67	53	11	16	—	9.4	5.8
Average cycle length (hours)	0	.20	.25	.33	.33	.85	1.5	5.5

paraffin and a plastic slip. After 24 hours at 25° C. the injured pupae, now with enhanced respiration, were returned to 10° C.: 24 hours later, respiratory measurements were performed at the low temperature.

As is evident in Table IV, the enhanced metabolism was accompanied by a conspicuous decrease in cycle length and burst volume, while in 5 of the 6 animals the interburst rate of carbon dioxide output increased.

In a second series of experiments, the animals after injury were maintained constantly at 25° C. Four days after the injury, the pupal metabolism was maximal and the release of carbon dioxide was continuous. However, after nine further days, the cyclic release of carbon dioxide reappeared as the metabolism decreased towards normal.

5. *Effects of changes in metabolism accompanying the larval-pupal and pupal-adult transformations*

The extremely high metabolism of the mature larva decreases precipitously during the larval-pupal transformation and the early weeks of pupal life (Schneiderman and Williams, 1953a). By the third or fourth week after the pupal moult the metabolism has fallen to the extremely low level characteristic of diapause. These

circumstances permitted a further route of approach to the influence of metabolic rate on the cyclic release of carbon dioxide. Observations on a typical animal are recorded in Table V; Figure 4 presents a semi-diagrammatic summary of results obtained from five individuals.

It is evident that carbon dioxide release is continuous throughout the prepupal period. Then, on the first day after the pupal moult, indications of cyclic release appear in the form of frequent bursts superimposed on a large continuous output of carbon dioxide. As the metabolic rate continues to fall, there is a corresponding decrease in the frequency of bursts and in the rate of carbon dioxide release between bursts. These trends continue until the metabolic rate stabilizes at the low diapause level four weeks after pupation.

When previously chilled pupae are placed at 25° C. to terminate the pupal diapause, the increase in metabolic rate after the onset of adult development is accompanied by a reversal of the above-mentioned changes. The carbon dioxide output gradually becomes more continuous and, finally, on about the third day of adult development, the carbon dioxide bursts disappear altogether. Even then, however, the cyclic respiration can be re-established by placing the animals at low temperature.

THE EFFECTS OF VARIATIONS IN OXYGEN AND CARBON DIOXIDE TENSION ON THE CYCLE OF CARBON DIOXIDE RELEASE

The preceding experiments reveal that the mechanism responsible for the discontinuous release of carbon dioxide is profoundly affected by metabolic rate. One would anticipate that an increase in metabolism, by increasing the utilization of oxygen and the production of carbon dioxide, would induce concomitant changes in the tensions of these gases in the respiring cells. This is especially true in an animal such as a silkworm pupa where respiratory movements seem to be absent and where the ventilation of the tracheal trunks apparently depends on gaseous diffusion. For these several reasons it seemed likely that the observed effects of metabolic rate on the cyclic release of carbon dioxide were attributable, in whole or in part, to changes in the "internal" tensions of oxygen and/or carbon dioxide. In order to test this possibility, the internal tensions of these gases were caused to vary.

1. *Effects of oxygen tension on the cyclic release of carbon dioxide*

The gaseous exchange of five diapausing pupae was first measured for 22 hours on successive days at oxygen tensions varying from 6 to 100 per cent of an atmosphere. In this and subsequent experiments employing gas mixtures, each vessel was flushed with about 25 volumes of the experimental gas. This procedure, in itself, commonly caused a slight prolongation of the succeeding cycle of carbon dioxide release; however, the effect was easily recognized and was taken into account in the computations.

The average rate of carbon dioxide output of diapausing *Cecropia* pupae at 25° C. appeared to be independent of oxygen tension when the latter was varied from 6 per cent to at least as high as 40 per cent of an atmosphere (*cf.* Schneiderman and Williams, 1954). Though failing to affect the over-all metabolism,

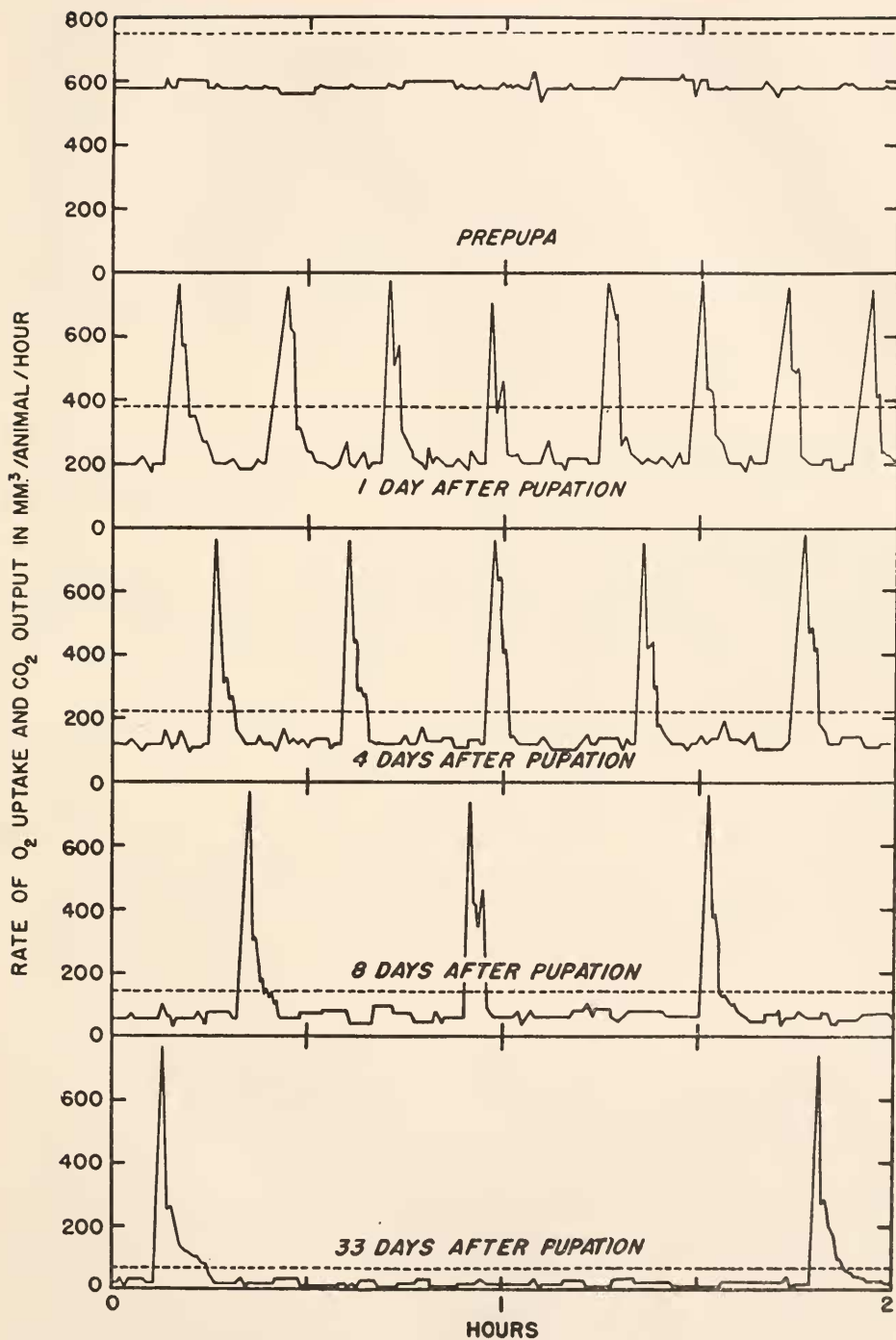


FIGURE 4. The rates of oxygen uptake and carbon dioxide output of a *Cecropia* silkworm during the larval-pupal transformation (semi-diagrammatic).

oxygen tension nevertheless induced striking changes in the cyclic release of carbon dioxide. Five animals were studied in detail with results summarized in Table VI. It will be observed that, as oxygen tension was decreased below the 21 per cent present in air, the interburst rate of carbon dioxide output increased; *i.e.*, a larger fraction of the carbon dioxide was released continuously. Finally, in 6 per cent oxygen, and occasionally, even in 15 per cent, all of the carbon dioxide was released continuously. When animals were placed in 6 per cent oxygen, it was noted that the rate of carbon dioxide output first increased markedly and then sank to a lower steady level only after several hours. Consequently, it is clear that the low oxygen tension triggered a prolonged period of carbon dioxide release and, in effect, terminated the cyclic release of carbon dioxide with an extremely long and voluminous burst. This same phenomenon has been noted by Buck *et al.* (1953) for pupae placed in nitrogen.

TABLE VI

*The effect of oxygen tension on the cycle of carbon dioxide release of five diapausing pupae**

Animal no.	Weight (grams)	Rate of O ₂ consumption (mm ³ /gm. live wt./hr.)	Rate of CO ₂ output (mm ³ /gm. live wt./hr.)					Interburst rate of CO ₂ output (mm ³ /gm. live wt./hr.)**				
			6%	15%	21% (AIR)	40%	100%	6%	15%	21% (AIR)	40%	100%
718(9)	3.35	12.8	11.0	—	9.8	10.3	—	11.0(100)	—	7.5(39)	4.0(39)	—
718(3)	3.87	13.2	12.1	10.9	11.4	—	—	12.1(100)	8.0(74)	5.7(50)	—	0(0)
718(2)	4.04	15.8	—	14.1	14.3	11.4	—	—	14.1(100)	8.2(57)	3.1(27)	2.6(18)
718(5)	4.42	16.5	14.7	14.9	13.1	12.1	—	14.7(100)	10.9(73)	5.3(91)	1.2(10)	0(0)
718(7)	3.85	16.8	10.4	—	9.4	14.0	—	10.4(100)	—	9.4(100)	3.9(28)	0.8(6)
(continued)			Average cycle length (hours)					Average burst volume (mm ³)				
718(9)			0	—	5.5	7.1	—	0	—	52	150	—
718(3)			0	6.9	5.3	—	7.5	0	76	116	—	—
718(2)			—	2.5	3.3	3.8	7.5	—	0	82	86	—
718(5)			0	8.8	4.0	3.0	5.0	0	158	130	149	170
718(7)			0	0	0	1.9	3.5	0	—	0	76	74

* Each datum is the average of 22 hours of measurements, save in the case of 100% oxygen where measurements were continued for only 7.5 hours.

** The percentage of over-all rate of carbon dioxide output (the interburst ratio) is recorded in parentheses.

It is of particular interest and importance to observe that oxygen tensions above that in air progressively decreased the proportion of carbon dioxide given off continuously. This fact is best appreciated in Table VI in terms of the large and progressive decrease in the percentage of carbon dioxide given off continuously. Indeed, in two of four pupae tested in 100 per cent oxygen, the ratio decreased to zero and there was no detectable release of carbon dioxide between bursts. One individual (No. 718 (7)), which exhibited only continuous release of carbon dioxide in air, showed a typical cycle of carbon dioxide release when placed in 40 per cent oxygen. While decreasing the interburst release of carbon dioxide, high oxygen tensions caused no systematic changes in the frequency or volume of the bursts; this was true save in pure oxygen where the burst frequency was always lower than in air, and where, in some cases, the duration of the bursts increased.

Four of the pupae in this experiment had also been studied in the low tempera-

ture study summarized in Table III. A comparison of the results in the two experiments reveals that exposure to 40 per cent oxygen was just as effective as 10° C. in decreasing the rate of carbon dioxide output between bursts. By contrast, the volume and frequency of the bursts were far more sensitive to low temperature than to high oxygen tension.

2. Effects of carbon dioxide tension on the cyclic release of carbon dioxide

The respiration of five pupae was measured on successive days over intervals of 15 to 25 hours at carbon dioxide tensions varying from 0 to 15 per cent with results summarized in Table VII.

TABLE VII
The effect of carbon dioxide tension on the cycle of carbon dioxide release of five diapausing pupae*

Animal no.	Weight (grams)	Rate of O ₂ consumption in air (mm ³ /gm. live wt./hr.)	Rate of CO ₂ output (mm ³ /gm. live wt./hr.)					Interburst rate of CO ₂ output (mm ³ /gm. live wt./hr.)**				
			0 (AIR)	5%	8%	10%	15%	0 (AIR)	5%	8%	10%	15%
675	6.62	8.9	7.1	5.1	5.4	6.0	5.3	0(0)	0.80(16)	2.5(46)	3.9(64)	4.8(90)
558	4.55	9.2	8.2	5.8	7.6	6.4	6.6	2.4(29)	1.8(31)	2.3(30)	—	5.9(90)
673	3.79	25.0	19.2	20.5	21.1	23.2	22.4	6.3(33)	14.7(72)	—	17.0(73)	20.0(90)
670	3.98	29.3	25.9	27.4	23.7	26.1	26.2	10.1(39)	14.7(54)	—	14.3(55)	22.6(86)
674	4.30	32.5	30.4	31.7	28.8	29.4	31.1	12.8(42)	19.7(62)	22.3(77)	23.4(80)	24.3(78)
(continued)			Average cycle length (hours)					Average burst volume (mm ³)				
675			6.5	5.4	.41	.32	.2	309	179	20	20	20
558			14.3	11.9	4.1	.2	.4	386	224	100	20	20
673			3.4	7.2	—	.37	.4	165	246	—	20	20
670			1.6	3.4	—	2.5	.36	116	170	—	116	20
674			3.5	3.3	.4	.31	.31	267	252	40	40	40

* Since it was not feasible to measure oxygen uptakes manometrically in the presence of high concentrations of carbon dioxide, in these calculations we assumed that oxygen uptakes remained constant as carbon dioxide tension varied. Since the average rate of manometer movement for each animal over a long interval did not vary with carbon dioxide tension, such an assumption appeared to be justified.

** The percentage of over-all rate of carbon dioxide output (the interburst ratio) is recorded in parentheses.

At ambient carbon dioxide tensions as high as 5 per cent, the effects on the cycle of carbon dioxide release were trivial: the interburst rate of carbon dioxide output increased slightly, but the burst frequency and volume failed to vary in any systematic way. Increase in carbon dioxide tension to 8 per cent further enhanced the rate of carbon dioxide output between bursts. But the most conspicuous effect of 8 per cent carbon dioxide was a sudden decrease in burst volume and an increase in burst frequency. Both these parameters now showed great irregularities, and in several cases a large and prolonged burst was given off within the first few hours and followed thereafter by a sequence of smaller bursts.

As the carbon dioxide concentration was increased above 8 per cent, more and more of the carbon dioxide was given off continuously. Though periodic irregu-

larities could still be detected, it became difficult or impossible to distinguish between a burst and an interburst. Only one pupa continued to give off large, regular bursts in 10 per cent carbon dioxide. As the carbon dioxide tension was increased to 15 per cent, the carbon dioxide output became almost continuous.

3. *Effects of spiracular occlusion on the cycle of carbon dioxide release*

Pupae of the *Cecropia* silkworm possess one pair of thoracic spiracles and six pairs of functional abdominal spiracles. These openings are the sole gateways to the tracheal system; consequently, their occlusion affords the simplest possible method of lowering the internal tension of oxygen and elevating the internal tension of carbon dioxide.

The over-all effects of spiracular occlusion were first examined in a series of 14 pupae by measuring the respiratory exchange before and after various numbers of spiracles had been sealed with melted paraffin. Occlusion of the spiracles had similar effects on the over-all uptake of oxygen and output of carbon dioxide. When all spiracles were sealed, the oxygen uptake and carbon dioxide release were reduced to less than 3 per cent; hence, virtually all the gas exchange of the pupa takes place *via* the spiracles. Occlusion of all six pairs of abdominal spiracles depressed the oxygen uptake and carbon dioxide output by one-third. By contrast, occlusion of the thoracic spiracles alone or the three posterior pairs of functional abdominal spiracles had trivial effects on the over-all gas exchange.

The sealing of the three posterior pairs of abdominal spiracles, while failing to lessen the uptake of oxygen, caused noticeable effects on the cycle of carbon dioxide release. In the four individuals studied in detail, there occurred an increase in the interburst rate of carbon dioxide output and a decrease in the burst volume; indeed, in one pupa the bursts disappeared completely. As might be anticipated, it was possible to reverse these effects to a considerable extent by increasing the ambient oxygen tension.

OXYGEN UPTAKE DURING THE CYCLE OF CARBON DIOXIDE RELEASE

In confirmation of the initial observations of Punt (1943, 1948, 1950) the experiments, up to this point, leave little doubt concerning the discontinuous output of carbon dioxide by diapausing *Cecropia* pupae. However, they likewise direct attention to the paradoxical fact that the oxygen uptake through the very same spiracles is apparently continuous.

Buck *et al.* (1953) suggested that the continuous uptake of oxygen might be an artifact attributable to a change in the volume of the pupa during the interburst period. Such a change in displacement, it was suggested, could result from a utilization of oxygen from the sealed tracheal system leading to a compensatory telescoping of the pupal abdomen. Manometric and volumetric respirometers are, of course, particularly susceptible to errors of this type, and any change in the insect's volume would necessarily be recorded as a net uptake of oxygen. For these several reasons, special attention was centered on the problem of oxygen uptake during the interburst period. Three independent experimental methods were developed as follows:

1. Divided chamber experiment

The object of the maneuvers now to be considered was to measure any volume change occurring in the motile collapsible segments of the pupal abdomen while simultaneously measuring the gaseous exchange of the entire pupa. For this purpose the three pairs of functional spiracles on the motile abdominal segments were sealed with melted paraffin. As diagrammed in Figure 5, a plastic diaphragm was then placed around each such pupa just anterior to the motile abdominal segments and sealed in place with melted paraffin. The three individuals treated in this manner continued to show cyclic release of carbon dioxide. Each pupa was then placed in a divided chamber capillary respirometer, adapted from that described by Fraenkel (1932) and diagrammed in Figure 5. The diaphragm separated the two 20-cc. chambers. The posterior chamber enclosing the abdominal segments was filled with water and coupled by a two-hole rubber stopper to a glass capillary calibrated in mm^3 units, the second hole being plugged with a rubber vaccine stopper. The water extended about half-way into the capillary at the outset. In this manner, the posterior chamber and its capillary were made

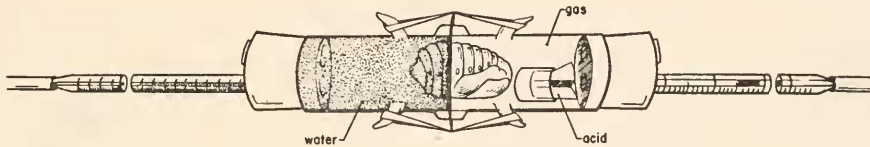


FIGURE 5. Divided chamber capillary respirometer. The diapausing pupa is sealed to a transverse plastic diaphragm so that the insect's anterior end is in the air-filled anterior chamber. The pupal abdomen (with spiracles sealed) is in the water-filled posterior chamber.

to function as a gauge to measure any changes in the volume of the motile abdominal segments.

The air-filled anterior chamber was similarly coupled by a two-hole rubber stopper to a glass capillary calibrated in 10 mm^3 units. A small paraffin-coated tube was placed in the anterior chamber to separate the insect from a roll of filter paper moistened with 0.3 cc. of HCl. In order to maximize the discontinuous release of carbon dioxide, a mixture of 40 per cent oxygen and 60 per cent nitrogen was flushed through the anterior chamber *via* the vaccine stopper. An index drop of colored detergent solution was then introduced by a long hypodermic needle into the anterior capillary. A short length of rubber tubing was attached to the tips of both measuring capillaries and the entire apparatus finally submerged in a constant temperature bath along with a suitable thermobarometer, the open ends of the rubber tubes being supported above the water level. Simultaneous measurements were made on the two capillaries in order to correlate changes in the displacement of the abdomen with changes in the gas volume attributable to respiration.

A typical experiment is diagrammed in Figure 6. The solid upper curve here records the volume changes of the anterior respiration chamber. The solid lower curve records the volume changes of the abdomen. The dotted line combines these two volume changes and charts the net volume changes that would

have been recorded if the animal were enclosed in a Warburg respirometer in the presence of acid. After 105 minutes, oxygen was injected to restore the original gas concentration and the experiment continued.

It is at once evident that the changes which were actually observed in the volume of the abdomen were extremely minor. In the experiment in Figure 6 three of the abdominal volume changes more or less correlate with carbon dioxide

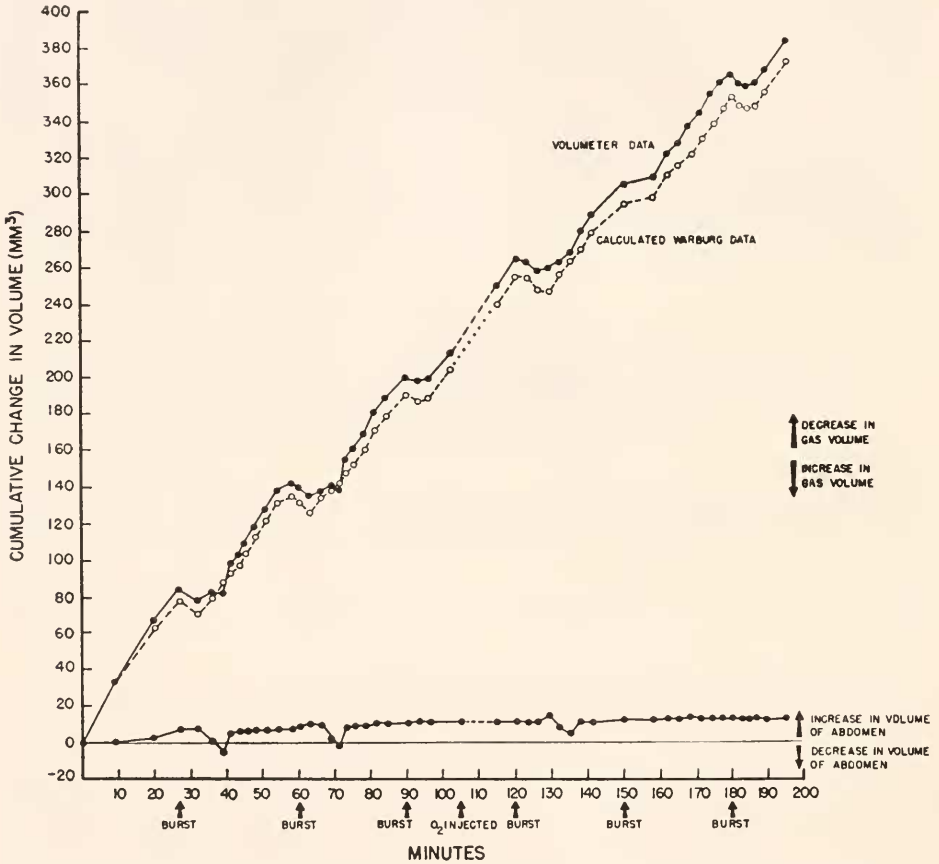


FIGURE 6. Volume changes of the pupal abdomen in relation to the cyclic release of carbon dioxide. See text.

bursts. However, bursts occurred on three other occasions without any detectable change in abdominal volume. Moreover, in all cases where changes in abdominal volume were recorded, they took place at least several minutes after the initiation of a burst and were of exceedingly short duration; namely, an abrupt slight decrease followed by an equally rapid increase. It is of particular interest to note that any decrease in abdominal volume was always accompanied by a corresponding increase in the volume of the anterior end. This fact accounts for

the smooth contours of the dotted curve in Figure 6 as contrasted to the irregularities in the upper solid curve. In short, we were unable to detect any net changes in the displacement of the pupal abdomen during the cycle of carbon dioxide release; evidently, the observed minor alterations in abdominal volume are to be accounted for in terms of the transfer of gas or liquid within the insect by slight changes in the tone of the abdominal muscles.

2. Projection studies

By an application of the projection technique of Plateau (1884) and Langendorf (1883), a pupa was placed on a firm pedestal in a dark-room and oriented in the path of the intense point-source of light from a 100-watt zirconium arc. The

TABLE VIII

The effect of immobilization on the cycle of carbon dioxide release

A					
<i>Immotile anterior pupal fragments</i>					
Animal no.	Weight (grams)	Rate of O ₂ consumption (mm ³ /gm. live wt./hr.)	Interburst rate of CO ₂ output (mm ³ /gm. live wt./hr.)	Average cycle length (hours)	Average burst volume (mm ³)
579(1)	3.38	30.5	3.6	0.35	85
581(1)	3.16	41	15.5	0.50	43
581(2)	3.20	44	14	0.60	60
579(2)	3.34	48	10.6	0.64	62

B					
<i>Pupae immobilized with wax "strait-jackets"</i>					
Animal no.	Weight (grams)	Rate of O ₂ consumption (mm ³ /gm. live wt./hr.)	Interburst rate of CO ₂ output (mm ³ /gm. live wt./hr.)	Average cycle length (hours)	Average burst volume (mm ³)
718(8)	4.86	20.6	9.6	9.9	178
718(6)	4.61	20.8	16.3	9.0	252

much enlarged shadow of the pupa was then viewed in profile on a white wall four feet away. Outline sketches of the shadow at frequent intervals revealed no detectable changes in the insect's profile as a function of time.

3. Experiments on immobilized pupae

By surgical techniques described previously (Williams, 1947) a series of four pupae were transected just anterior to the motile abdominal segments. Each of the immotile anterior fragments was then sealed with melted paraffin to a plastic slip. Several weeks later, after the injury-induced metabolism had subsided, the measurements of respiratory exchange demonstrated the usual cycle of carbon dioxide release along with a continuous uptake of oxygen (Table VIII, part A).

A second procedure involved the immobilization of the abdominal segments of normal pupae by means of wax "strait-jackets." Pupae were anesthetized and

strips of melted paraffin applied to their abdominal segments until the segments were completely immobile and incapable of telescoping. Care was taken to avoid any damage to the spiracles. Measurements on two such pupae after immobilization are presented in Table VIII, part B. The discontinuous nature of the carbon dioxide output persisted, as did the apparently continuous oxygen uptake.

Consequently, it is evident that the presence of the motile abdominal segments is not prerequisite for the phenomena under consideration.

DISCUSSION

The results just considered confirm the reality of the carbon dioxide bursts and delineate the factors which affect their magnitude and frequency. All conditions which alter the internal tensions of oxygen or carbon dioxide influence the kinetics of the cyclic release of carbon dioxide. Table IX summarizes the direction of

TABLE IX
Summary of the effect of various factors on the cycle of carbon dioxide release

Factor	Metabolic rate	Interburst rate of CO ₂ output	Cycle length	Burst volume
Increasing pupal mass	Variable	Decreases	Increases	Increases
Increasing temperature	Increases	Increases	Decreases markedly	Decreases
Integumentary injury	Increases	Increases	Decreases	Decreases
Increasing O ₂ tension (6% to 100%)	No effect	Decreases markedly	Variable	Variable
Increasing CO ₂ tension (0 to 15%)	No effect	Increases	Variable	Variable
Larval-pupal transformation	Decreases	Decreases	Increases	Variable
Pupal-adult transformation	Increases	Increases	Decreases	Decreases
Occlusion of three abdominal spiracles	No effect	Increases	Variable	Decreases
Occlusion of all spiracles	Maximal decrease	None	None	None

these influences. The data suggest that within the insect the normal stimulus for a carbon dioxide burst is either low internal tension of oxygen, high internal tension of carbon dioxide, or a combination of the two. Moreover, bursts appear to be correlated with low metabolic rate. Therefore, in other groups of insects which, because of their high metabolic rate, release their carbon dioxide continuously at ordinary temperatures, it is likely that bursts can be induced by exposure to low temperature. Also, the greater volume of the burst at low temperatures suggests that the threshold concentration of carbon dioxide necessary to trigger a burst is increased at lower temperatures.

The manometric description of the cycle of carbon dioxide release agrees substantially with the results which Punt (1950) obtained by the diaferometric method. Unfortunately, since Punt's experiments were qualitative and involved only measurements of carbon dioxide output, we cannot make any quantitative comparisons. However, even on a qualitative basis, there are a few minor differences between his results and ours. Whereas we recorded an apparently constant rate of carbon

dioxide output between bursts, Punt's data show a period of increased carbon dioxide output prior to each burst. But, since the units along his ordinate are not given, we cannot ascertain the amount of carbon dioxide released in this pre-burst period. Under our experimental conditions, we were unable to detect manometrically a pre-burst increase in carbon dioxide. It is likely, therefore, that Punt's pre-burst increases in carbon dioxide are of small magnitude.

A consideration of the effects of low temperature on the cyclic release of carbon dioxide provides insight into the mechanism underlying the bursts. By comparing the carbon dioxide release curves of animals at 10° and 25° C., we see that, for bursts of equal volume, the rate of evolution of carbon dioxide during the burst is substantially the same at the high and the low temperature. The low temperature coefficient, in itself, suggests that the kinetics of the burst is governed by one or more rate-limiting processes of a physical rather than a chemical character.

By contrast with the cyclic release of carbon dioxide, the uptake of oxygen appears continuous even at low temperatures and under conditions of low metabolic rate. The results of the projection studies and of experiments with the divided chamber and with isolated pupal fragments support this conclusion. Also, as the following calculations show, measurements of tracheal volume add further confirmation.

Vacuum extraction of the gases in the tracheal system and dissolved in the tissue fluids revealed the tracheal volume of *Cecropia* pupae to be considerably less than 10 per cent of the total volume of the insect. Thus, in the absence of a special oxygen-absorbing blood pigment, a *Cecropia* pupa weighing 5 grams and having a specific gravity of approximately 1, could store within its tracheal system not in excess of 100 mm³ of oxygen, and dissolved within its tissue fluids less than an additional 100 mm³ of oxygen. In short, the total "storage" of oxygen could scarcely exceed 200 mm³ and is probably much less. However, such a pupa may consume more than 600 mm³ of oxygen between bursts; that is, more than three times as much oxygen as could be stored under optimum conditions. These facts give assurance that oxygen must enter the insect in substantial quantities between bursts.

The manometric data reveal the magnitude of this oxygen uptake during this interburst period: at 25° C., oxygen enters the spiracles at a rate five to twenty times greater than that at which carbon dioxide makes exit; at 10° C., the difference in rate may reach 100-fold. Any theory which accounts for the discontinuous release of carbon dioxide must also provide for the continuous uptake of oxygen and for the quantitative aspects of the cycle.

It is illuminating to calculate the respiratory quotients one would obtain at 10° and 25° C. if the burst phenomenon were not recognized and the interburst carbon dioxide output employed in calculating respiratory quotients. Thus, at 25° C. the average "interburst R.Q." for the first group of animals in Table III is 0.43 with a range of 0.32 to 0.56. At 10° C. for this same group of animals the average is 0.25 with a range of 0.12 to 0.42, while for the second group it is 0.13 with a range of 0.01 to 0.29. The apparent "systematic change in R.Q. with temperature" is clearly an artifact attributable to the discontinuous release of carbon dioxide. Neglect of this factor doubtless accounts for the peculiarly low R.Q.'s usually reported for diapausing insects.

In the hard-bodied pupal insect we have seen that the spiracles provide the sole

gateways to the tracheal system and that the sealing of these orifices brings respiratory exchange substantially to a standstill. Consequently, attention focuses on the role of the spiracular mechanism in relation to the cyclic release of carbon dioxide. This matter will be considered in the paper that follows.

SUMMARY

1. The respiration of diapausing *Cecropia* pupae is remarkable in that metabolic carbon dioxide is retained within the insect and released during brief periods as "bursts." This finding confirms Punt's prior observations on several species of diapausing insects.

2. At 25° C. in 14 pupae with an average weight of 4.7 grams, bursts of carbon dioxide occurred on the average of once every 7.3 hours and the burst volumes averaged 271 mm³/animal. The average interburst rate of carbon dioxide output was 2.7 mm³/gm. live wt./hr., *i.e.*, about one-sixth the average rate of oxygen uptake.

3. The utilization of oxygen, unlike the release of carbon dioxide, shows no discontinuities and "direct" Warburg manometric procedures reveal an apparently continuous uptake of oxygen.

4. Several independent lines of experimentation confirm the manometric observations that oxygen uptake by the pupa is continuous and non-cyclic and direct attention to the surprising fact that, in the interburst period, oxygen may enter the insect at many times the rate at which carbon dioxide makes exit.

5. Virtually all respiratory exchange ceases after the spiracles are sealed with wax; the tracheal system and spiracles are therefore the site of both the discontinuous release of carbon dioxide and the simultaneous continuous uptake of oxygen.

6. The cycle of carbon dioxide release is a function of metabolic rate and therefore of temperature. At low metabolic rates the bursts are accentuated. Thus, at 10° C. carbon dioxide is given off only once in several days and the interburst rate of carbon dioxide output may be but 1/100th the rate of oxygen uptake. If the insect's metabolism is increased by integumentary injury, or development, or increase in environmental temperature, the bursts become more frequent and the continuous release of carbon dioxide more pronounced. The burst phenomenon usually disappears when the oxygen uptake rises beyond 160 mm³/gm. live wt./hour.

7. The bursts also vanish when the external oxygen tension is decreased below 15 per cent or the external carbon dioxide increased above 10 per cent. The interburst rate of carbon dioxide output is especially sensitive to oxygen tension and in pure oxygen the interburst carbon dioxide output may become undetectable.

8. For bursts of equal volume, the rate of release of carbon dioxide during the burst is substantially the same at 10° and 25° C., signifying that the rate-limiting processes in the rapid release of carbon dioxide are of a physical character with low temperature coefficients.

9. The discontinuous release of carbon dioxide is apparently a widespread phenomenon in diapausing pupae. This fact complicates determinations of respiratory quotients and is evidently responsible for the extremely low and apparently erroneous values reported for diapausing pupae.

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